Remarks

Applicants hereby affirm the telephonic election of a restriction group of claims made on July 5, 2000, as described by the Examiner in the Office Action mailed July 18, 2000. Claims 1-27, 50 and 52 are pending in the application. Applicants reserve the right to pursue withdrawn or canceled claims in a related application having the same priority date as the present application.

Claims 1-3, 11-12, 16-17, 22, 24-27, 50 and 52 are here amended. Support for the amended claims can be found in the original claims, and in the description as follows. Claim 1 as amended is directed to a nucleic acid composition for muting expression of a gene in a population of cells, for example, 10^6-10^7 cells (for support, see p. 8, line 8, and lines 24-25). Claim 2 is directed to such a composition wherein the gene to be muted is a chromosomal gene, located on the genome of the cell (see original claim 2, and p. 10, lines 10-13). Claim 3 is directed to a nucleic acid for muting expression of a gene in cells selected from the group of cells of a cancer, cells associated with an autoimmune condition, and cells having a gene of a pathogen (see original claim 3, and p. 10, line 29-p. 11, line 1). Support for amended claim 24 can be found in Fig. 3, panel B footnote (f), and on p. 8, lines 24-25. Support for the other amended claims can be found in the original claims, and throughout the description. The amended claims use terms of art of the fields of cell biology and genetics.

The attorney docket number, original docket number 2281/102, has been changed to new attorney docket number 2498/101 because of withdrawal of previous client Christopher P. Adams. As executed assignments from the inventors to the previous client were not filed, it is believed that no additional formal papers need be added to the pending application concerning this change.

The claimed invention

Applicants believe that it would be helpful to the Examiner to briefly summarize the main points regarding the invention defined by the claims amended herein before addressing points raised by the Examiner in the office action.

The invention of claims as amended herein is based on a phenomenon never before described in any cells: reducing expression of an endogenous chromosomally located gene by exogenously supplied homologous transgenes which have sequence homology to part or all of the

target endogenous gene. The lexicographically new term, "muting," defined in the present application on p. 10, lines 10-13, means "a method of using a transient non-integrated transgene to reduce expression of an endogenous gene" (p.10, lines 10-11). This definition applies to the description and to the claims (p. 9, lines 27-29). Muting is different from earlier observed processes of silencing and co-suppression in plants, fungi, and *Drosophila* (see p. 1, lines 20-p. 2 line 6 of the present application).

Muting of expression of a gene is not a rare genetic heritable phenomenon (for example, a one in a million proper double cross-over event to produce a deletion/insertion such as a knock-out mutation, see Capecchi, M. p. 57, left column, lines 3-5). Rather, muting is a regulatory phenomenon, that reduces expression of a gene (see p.4, lines 18-19, for example, by suppression of mRNA because of the presence of transiently maintained exogenous foreign genes, in all cells receiving such DNA by any DNA transfer process such as transfection, transformation, lipofection, etc. (see the present application p. 8, lines 6-7 p. 20 lines 14-15 and lines 17-29).

During a transient transfection, the number of plasmids maintained in the cell, which is initially as high as a few thousand per cell, is diluted by growth of the cells over several days (p. 13, lines 21-23). The vast majority of plasmids in each cell, however, remain extra-chromosomal. i.e., are not integrated into the chromosome but are maintained in a substantially transient condition in a majority of the transformed cells (p. 11 lines 9-12). Hence muting is not a genetic event involving either a random single or a precise double cross-over homologous recombination event into a chromosomal site.

The inventors have made the surprising finding that the exogenously supplied "transgene," while present in thousands of copies and supplied with its own promoter (p. 23, line 25) is not expressed (p. 22, lines 1-5). Further, the exact same plasmid, when integrated into the cellular genome, was found by others to be expressed (p. 2, lines 22-25; p. 22 lines 5-10). Thus muting of the endogenous gene by the transgenes (to about 7% of control untreated levels, p. 22 lines 27-29) requires that the plasmid which contains the muting nucleic acid be found as free circular DNA, a transient state. Gene expression from this vector requires (p. 22, lines 11-14) integration into a chromosome, resulting in a process which is the opposite of gene muting.

The muting effect of a gene in a population of cells is therefore much more rapidly and efficiently produced than a knock-out mutant animal, since it does not require all the genetic

breeding processes necessary to produce a knock-out mutant. No selection step is performed, let alone a second "negative" selection. To obtain a knock-out mutation, a selection for a drug-resistance marker located between two adjacent regions of a chromosomal gene is required. Further to obtain a knock-out mutation, selection against acquisition of an outside marker, for example the tk marker, is required to obtain true targeting into the gene of choice rather than random insertion into the chromosome. These steps are not necessary for gene muting. Claims directed to embodiments of the present invention which are compositions for muting expression of a gene (claims 1-10, 50 and 52) do not describe such selective marker, because they are not necessary. Claim directed to embodiments of the present invention which are methods of muting a gene (claims 11-27) do not contain a step of selecting for a drug-resistance determinant that is an insertion into a targeted gene nor a step of counter-selecting to eliminate recombinants having an outside drug-resistance marker. These features mean that gene muting can be used to efficiently and quickly evaluate what a knock-out phenotype of a cell would be, without actually having to construct a knock-out mutation.

The written description is enabling and the inventor had possession of the claimed invention

Examiner's rejection of claims 11-27, 50 and 52, based on lack of written description to required to enable one skilled in the art to practice the invention, 35 U.S.C. §112 ¶1, is improper because the description at the time it was filed provided compositions and methods for muting an endogenous gene in a population of animal cells, and also provided the technology necessary for one skilled in the art to practice the invention that was readily accessible and routinely performed at the time the invention was made.

Claims 11-27, 50 and 52 were rejected under 35 U.S.C. §112 1st paragraph, as allegedly not enabling knock-out mutations, and as allegedly not enabling gene therapy. It was not the intent of the Applicants to enable knock-out mutations or gene therapy, however, because the invention, as defined by the original claims and by the present claims, is directed to compositions and methods for *muting* gene expression in animal cells.

As described above, the term "muting" is defined in the application and the meaning of muting is distinct from a mutating by knockout techniques. As defined for both the description and claims (see application p. 9, lines 27-29), "muting" means a method of using a transient non-integrated transgene to reduce expression of an endogenous gene" (p.10, lines 10-11). This term,

which appears in the only independent claims presented here, namely claims 1 and 11, rules out making a mutation by knockout techniques or otherwise. Hence, a rejection for lack of enablement of knockout techniques is improper.

In contrast, it is well-known that an integrated transgene is essential for creating a knock-out animal (see Capecchi, Figure legends on pp. 53-55). The term "muting" is directed to use of a transient non-integrated transgene, which is inconsistent with requirements for creating a knock-out animal. Because of the meaning of "muting", independent claim 1, and claims dependent on claim 1, define a scope that exclude creating knock-out animals.

The Examiner further states that "claims 11-27 read on a method of creating a knockout mammal as interpreted in light of the teachings of the application (see application page 3 line 6, also page 4 lines 18-20). Because the term "muting" is also used in claims 11-27, the claims exclude creating a knockout animal. Indeed, in page 3, line 6 of the application, muting of expression is described as an "...alternative to engineering a knock-out animal..." (emphasis added). In other words, the embodiments of the present claims are something other than engineering a knock-out animal, although having potentially similar effects on the phenotype of the resulting muted cells. Page 4, lines 18-20 of the application refers to "...a muting nucleic acid that reduces expression of an endogenous target gene...".

The Examiner alleges that "it would be unpredictable to expect to mute expression of any endogenous gene in a mouse without integration or stable expression of a muting transgene...". In contrast, the application demonstrates muting of expression of a endogenous gene by providing many working examples. (See Example 6, p.21 lines 24-p.22 line 6; Example 7, p.22 line 28-p. 23 line 19; Example 9, p. 26 lines 5-9, p. 27 lines 1-5, and other locations.) Further, because muting is premised on a mechanism different from knockout techniques, it does not require or utilize integration or stable expression. Indeed, "to mute expression of an endogenous gene without integration or stable expression of a muting transgene" is a novel and non-obviousness feature of embodiments of the present invention. The novelty and non-obviousness of the embodiments of the present invention are discussed below (see sections concerning prior art by Capecchi), as distinctions from gene targeting and engineering to produce knock-out animals. Furthermore, the application shows that substantial muting "without integration or stable expression" can be achieved, and is thereby not unpredictable to expect.

The Examiner also rejects the claims as allegedly failing to enable gene therapy, because the examiner believed that the claims read on gene therapy of any and all diseases using a pharmaceutical composition. The present application does not claim that the muting method be used to treat any disease. Even given their broadest reasonable interpretation, the claims do not pertain specifically to a therapy method, although the claims do not exclude the possibility that the claimed methods can be used in therapy in the future.

Accordingly, Applicants believe that the present application does not read on either knock-out mutant animals, or depend on methods of gene therapy. It is therefore irrelevant whether the present application provides enablement for either knock-out mutants or gene therapy. For the reasons stated above, Applicants respectfully request that the 35 USC §112 1st paragraph rejections be withdrawn.

Amended claims 1-27 point out and distinctly claim the embodied inventions

Applicants have amended claims 1-3, 11-12, 16-17, 22 and 24-27, in response to the Examiner's rejections. (See Remarks section above.)

Claims 1-27 are rejected under 35 USC §112 2nd paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the invention. The Applicant respectfully disagrees with the Examiner.

The Examiner alleges that the term "muting" does not convey a clear meaning, and thought it is unclear whether the expression of the "muted" gene is completely abolished or only reduced. Applicants assert that the description clearly defines muting as a reduction in expression, rather than abolition of gene function.

In the application, the term muting is defined as "a method of using a transient non-integrated transgene to reduce expression of an endogenous gene, for example located on the genome of a cell, the endogenous gene having a portion of substantial homology to the transgene." From this definition, the Applicants believe that the term muting does convey a clear meaning. Because the meaning of muting is clear, it is also clear that it is the expression of the muted gene that is quantitatively reduced. See p. 30, lines 9-18, showing muting of expression as reduction to, for example, 50%, or to 70%, of the non-muted control.

The Examiner alleges that the term "substantially homologous" does not convey any clear meaning. "Substantially homologous" is widely used in the field of biological sciences to

describe the similarity between two sequences of DNA (or RNA or polypeptide). The meaning of "substantially homologous" is clear to one ordinarily skilled in the art. Furthermore, "substantially homologous" is also often used in US patent practice to describe the similarity between two non-identical but closely related nucleic acid sequences, e.g., U.S. patent numbers 4,766,073, 5,827,693, and 6,060,447.

The Examiner also claims that the term "substantially integrated" does not convey any clear meaning. Applicants agree with the Examiner that if the term referred to a single plasmid, then this plasmid could either be integrated or not integrated, so that "substantially integrated" would have no clear meaning for such a plasmid. The claim 27 as amended, however, pertains to a preparation of plasmids, rather than to a single plasmid. A preparation of plasmids is used is this case, in which the recipient population of cells is transformed at high copy number with a type of plasmid which is designed to be only transiently maintained. In that context, "not substantially integrated" means that the vast majority, if not all, plasmids in the cells are not integrated. In fact, it has been generally found that to be integrated at any measurable frequency, a plasmid must be specifically engineered (see sections below on novelty and non-obviousness). Thus, the term "not substantially integrated" conveys a clear meaning that is required by the context.

Accordingly, the Applicants believe that the terms of the claims, as amended, convey clear meanings. The Applicants respectfully request that the 35 U.S.C. §112 2nd paragraph rejections be withdrawn.

The subject matter of the claims is not anticipated in light of the cited art

The claimed invention described in claims 1-17 and 22-24 nor their equivalent is not anticipated by Capecchi, M., March 1994, *Scientific American* p. 52-59. Case law states that "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). This reference does not anticipate the claimed invention of the claims nor equivalents, because the art described by Capecchi, is not the same as elements of any of the claims.

Capecchi reviews the background and technology of targeted gene replacement (gene knock-out mutant construction), including an overview of methods for producing and uses of

mouse knock-out mutants. Of note are the structures of suitable vectors for targeting a chromosomal gene, and the steps that are required to obtain the necessary double cross-over recombination event which deletes a piece out of the middle of the coding portion of the gene, and inserts instead a selectable drug-resistance determinant (see Capecchi, p. 53-56). Capecchi shows that the suitable vector has "mutated region" of the chromosomal gene to be targeted, and that the "mutated gene replaces [the] normal version in cellular DNA" (see Capecchi's Fig. on p. 53). The mutated gene is further described by Capecchi as a gene:

> inactivated by insertion of the *neo*^{r} gene (green) into a protein coding region (blue)...as a marker to indicate that the vector DNA took up residence in a chromosome. The vector has also been engineered to carry a second marker at one end: the herpes *tk* gene..." [Capecchi p. 54]

Capecchi further shows that the drug resistance markers are useful to:

> isolate cells carrying a targeted mutation, workers put all the cells into a medium containing selected drugs...the only cells that survive and proliferate are those harboring the targeted insertion...[Capecchi p. 55]

More importantly, Capecchi points out that "[r]egrettably, such targeted replacement occurs only in a small fraction of the treated cells" (p. 56, right hand column, last paragraph), and continues,

> More often, the targeting vector inserts randomly at non-matching sites or fails to integrate at all. Approximately one in a million treated cells has the desired replacement. To greatly simplify the search for that cell, we make use of two 'selectable markers' which are introduced into the targeting vector from the start. [Capecchi p. 57]

Thus Capecchi uses these markers to get hold of the rare clone of the truly targeted gene, among the much larger group of clones having the vector randomly inserted by a single cross-over event, into the chromosome.

In contrast to all of these genetic probabilistic considerations that Capecchi describes for gene targeting, the nucleic acid compositions of present claims 1-10, and the methods of claims 11-17 and 22-24, do not require a vector that carries a targeting gene which has been specially mutated to have a selectable marker, i.e., a drug-resistance determinant, inserted into the protein coding regions between two other pieces of the gene. Applicants' muting nucleic acid requires instead only a sequence which is homologous to an endogenous sequence, i.e., a chromosomal

sequence, in the gene which is to be muted. No selection of infrequent or rare clones is required. Further, Applicants' compositions do not have a second drug-resistance marker for selection against insertion of DNA outside of the mutated gene, and Applicants' methods do not describe any such selection steps. Applicants' inventions as embodied in the present claims are compositions and methods of muting an endogenous gene using a transient non-integrated transgene to reduce expression of the endogenous gene (p.10 of description, lines 10-13). Capecchi's compositions are not transient, and do not affect expression of a gene, but only mutate that gene by disrupting it. Capecchi is clearly not the same as Applicants' inventions of the amended claims, nor equivalents.

For at least the above reasons, the Capecchi reference does not anticipate the invention of claims 1-17 and 22-24. Applicants respectfully request that the rejection under 35 U.S.C. §102(b) be withdrawn.

The subject matter of the claims would not have been obvious in light of the cited art

Rejection of claims 1, 8-10, 12, 17-21 and 25-27 in light of Capecchi, M. (March 1994 *Scientific American*, p. 52-59) on the basis of 35 U.S.C. §103(a) is improper. To establish a *prima facie* case of obviousness, at least two criteria must be met: first, there must be some suggestion or motivation, either in the reference or in the knowledge available to one of ordinary skill in the art, at the time the invention was made, to modify the reference; and second, the prior art reference must teach or suggest all the claim limitations. (Manual of Patent Examining Procedure, 2:2143). The Capecchi reference as shown below satisfies none of these criteria.

As stated in *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991), ". . . .both suggestion and reasonable suggestion of success must be found in the prior art, not in Applicant's disclosure."

There is no suggestion or teaching in Capecchi of the elements of the invention defined for example by claims 1 and 11, muting of an endogenous gene, which is defined in the description as using a transient non-integrated transgene to reduce expression of the endogenous gene (see p. 10 of the description, lines 10-13). The term transient when used to describe a plasmid is a term of art, well-known in cell and molecular biology. See, for example, Ausubel et al., "Short Protocols in Molecular Biology," 3rd Ed., p.9-5, which states, "This unit contains two

methods for calcium phosphate-based eukaryotic cell transfection that can be used for both transient and stable (*Unit 9.5*) transfections." See also Unit 16.13 of the same volume, entitled, "Transient Expression of Proteins using COS Cells." Both sections are attached hereto, for the Examiner's convenience.

Capecchi does not teach or suggest transient transfection, rather only stable integration of a gene targeting vector specially engineered for knock-out purposes of a specific gene. The backbone for such a specially engineered gene targeting vector can be obtained commercially, for example, see p.274-275 of the New England Biolabs catalog, a copy of which pages is attached hereto for the Examiner's convenience. This gene targeting vector contains two multiple cloning sites (MCS I and MCS II) for inserting different pieces of the gene to be targeted. The multiple cloning sites span a drug-resistance determinant (neo) which has its own promoter. This highly specialized vector, a commercially available version of the type of vector described by Capecchi, does not teach nor suggest a vector that is transient, as required by the inventions embodied in the presently pending claims. As Capecchi does not describe any such transient vector, let alone suggest the success the using a transient vector to obtain muting of a gene, the invention of the present claims is not obvious in light of Capecchi. Use of a transiently maintained vector to obtain muting of an endogenous gene would not have been obvious to one of ordinary skill in the art at the time the inventions of the present claims were made, reading Capecchi.

The invention of claims 1 and 11 does not have the following elements required by Capecchi: micro-injection of the knock-out vector into embryonic stem cells (ES cells), injection of such transfected ES cells into a mouse, or selection using neomycin for knocked-out genes having the insert, nor counterselection such as with gancyclovir against insertion of an outside marker, such as *tk*. Omission of any one of these steps, let alone all of them, would not have been obvious to one of ordinary skill in the art at the time the inventions of the present claims were made, reading Capecchi.

Practicing the inventions of claims 1 and 11 makes possible embodiments that reduce but do not eliminate gene expression. The muting phenomenon is illustrated in Example 9, for example, which states, "the endogenous procollagen gene was suppressed by 50%, compared to untransfected cells (Figure 3, compare lanes 15 and 16)." See p. 25, lines 11-12. Capecchi makes

no mention of such quantitative reduction of gene expression, but is limited to the all or nothing phenomenon of gene knock-out, which when successful eliminates that gene by deletion/insertion genetic mechanisms. Muting of gene expression in an animal cell would not have been obvious to one of ordinary skill in the art at the time the inventions of the present claims were made, reading Capecchi.

For any of the reasons above, Capecchi would not have taught or suggested to one of ordinary skill in the art at the time the invention of the subject matter of claims 1, 8-10, 12, 17-21 and 25-27 was made, and therefore this reference would not have motivated one skilled in the art to make these inventions or equivalent inventions.

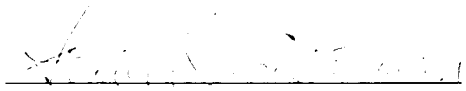
As the inventions embodied in the present claims were not obvious in light of Capecchi, Applicants respectfully submit that rejection of claims under 35 U.S.C. §103(a) be withdrawn.

Summary

In view of the foregoing amendments and remarks, Applicants submit that the claims are now in condition for allowance. Early and favorable reconsideration of the application is therefore respectfully solicited.

It is believed that a two month extension of time is required, Applicants hereby petition for same and request that any additional extension or other fee required for the timely consideration of this application be charged to Deposit Account No. 19-4972.

Respectfully submitted,



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